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SYNTHESIS OF TWO ESTRADIOL-IMIDAZOLE *C*-RIBONUCLEOSIDE HYBRID COMPOUNDS EXHIBITING INHIBITORY EFFECTS AGAINST TYPE 1 17β-HYDROXYSTEROID DEHYDROGENASE

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Abstract – Novel estradiol-imidazole *C*-nucleoside hybrid compounds **4a** and **4b**, which have C4-linked C_0- and C_2-imidazole ribonucleosides as adenosine mimics and amide bond linkers, were designed and synthesized based on EM-1745, an inhibitor of type 1 17β-hydroxysteroid dehydrogenase (17β-HSD1). Compounds **4a** and **4b** were also tested as enzyme inhibitors.

INTRODUCTION

Breast cancer is one of the most common cancers diagnosed among women and approximately 60% of breast cancers are hormone-responsive.[1] Estradiol (E_2), the most potent female sex hormone, stimulates the growth of mammary tumors[2] and endometriosis[3] by activating the estrogen receptor. One treatment approach for this type of cancer is to decrease the level of E_2 by inhibiting one of the enzymes involved in its biosynthesis.[4,5] Among those enzymes, type 1 17β-hydroxysteroid dehydrogenase (17β-HSD1) catalyzes the last step in the process of biosynthesis of E_2, as illustrated in Figure 1.[6,7] As this enzyme, utilizing the cofactor NAD(P)H, reduces the C17 ketone of estrone (E_1) into E_2,[8] 17β-HSD1 inhibitors are regarded as promising new agents for estrogen-induced diseases.[9] Poirier and co-workers recently developed E_2-adenosine hybrid compounds as a new type of 17β-HSD1 inhibitor (Figure 2).[10,11] The compounds were designed to exhibit affinity for both substrate (E_1 or E_2) and cofactor [NAD(P)H] binding domains of the enzyme.[11a,12] The most potent hybrid inhibitor is EM-1745 (**1** : IC_{50} = 52 nM),[10] in which E_2 is linked to the adenosine moiety via a 16β-oriented eight-CH_2 ester spacer. Crystal structure analysis of a complex of EM-1745 and 17β-HSD1 led to the identification of a series of hydrogen bonds

formed with E_2 (O3/His221, O17/Ser142, and O17/Tyr155) and adenosine (NH_2/Asp65 and OHs/Ser11) moieties.[10,11a,12] They also reported many simplified inhibitors containing **2** to improve the bioavailability of EM-1745,[13] in which aniline moieties bearing carboxylic acid function were used as adenosine mimics with 13 methylene linker (Figure 2). Although the enzyme inhibitory effects of **2** were less potent than those of EM-1745, the aniline structure of **2** showed flexibility on the adenosine moiety of EM-1745.

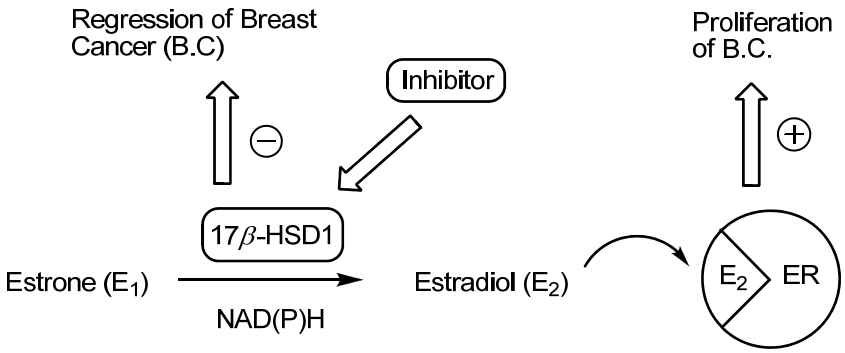


Figure 1. Role of 17β-HSD1 in the synthesis of E_2 and an approach to treat breast cancer using 17β-HSD1 inhibitor

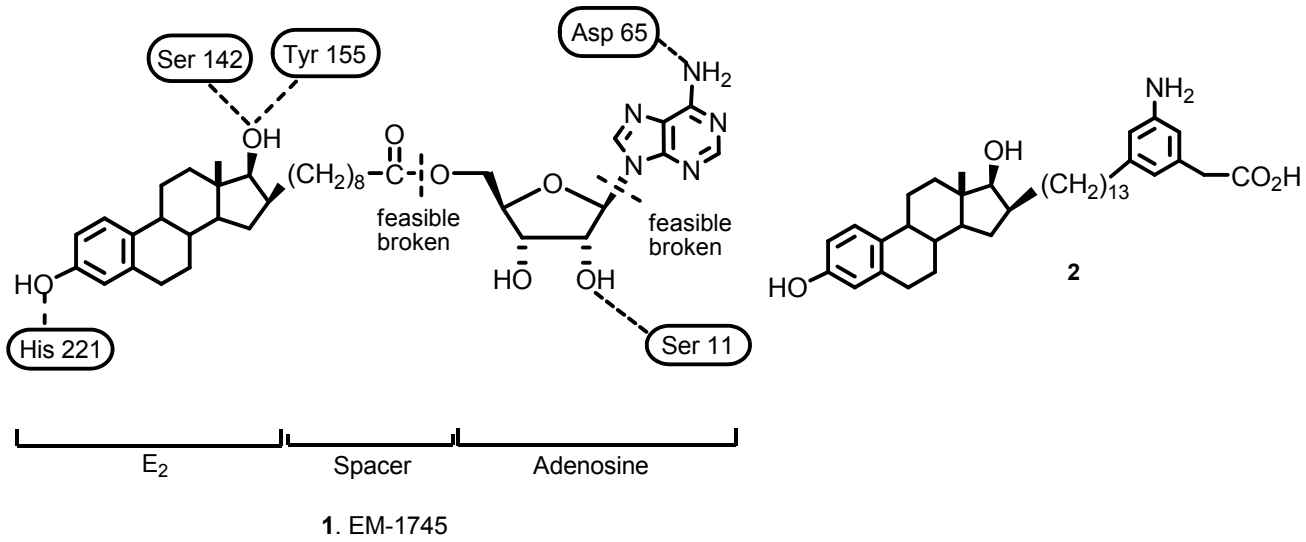


Figure 2. EM-1745 (**1**) and its main interactions with 17β-HSD1 and simplified hybrid inhibitor **2**

In our systematic studies on application towards novel bioactive compounds using imidazole *C*-nucleosides, we have recently reported that C4-linked (C_0)- and two-carbon (C_2)-elongated-imidazole ribonucleosides **3a** (n = 0)[14,15] and **3b** (n = 2)[16] are incorporated into the active sites of ribozymes to probe their role in the acid-base catalysis of the ribozymes (Figure 3).[17,18] This chemogenetic approach has

demonstrated the importance of particular adenine and guanine bases in the catalytic mechanism of ribozymes, showing that the imidazole-substituted ribozymes are active in both cleavage and ligation. The results suggested that the *C*-nucleoside **3a** and its C_2-elongated homologue **3b** could be used as structural mimics of adenosine or guanosine having purine bases.[16]

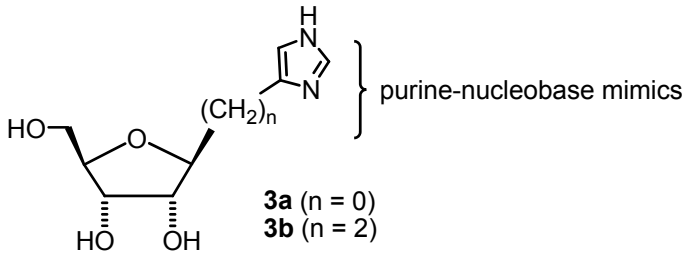


Figure 3. Imidazole C_0- and C_2-nucleosides **3a** and **3b** as purine-base mimics

In this context, we envisioned that the adenosine moiety and ester linkage in EM-1745, which were susceptible to hydrolysis, could be replaced with imidazole *C*-nucleoside and amide bond, respectively.[19] Further, the endocyclic amine function of the imidazole had possibility to form hydrogen bonds with Asp65 of 17β-HSD1, similar to the 6-amino group of adenine in EM-1745 (Figure 2). We herein report the synthesis of new E_2-imidazole *C*-nucleoside hybrid compounds (**4a** and **4b)** and the preliminary evaluations for 17β-HSD1 (Figure 4).

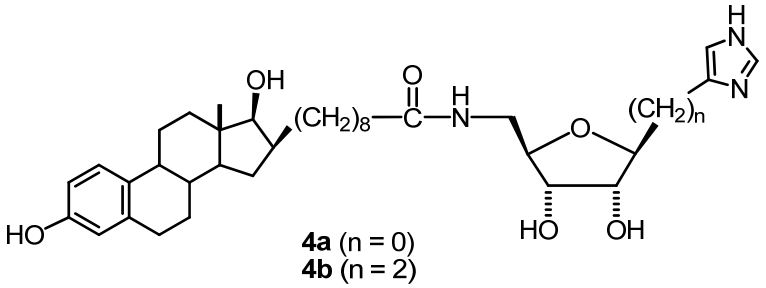
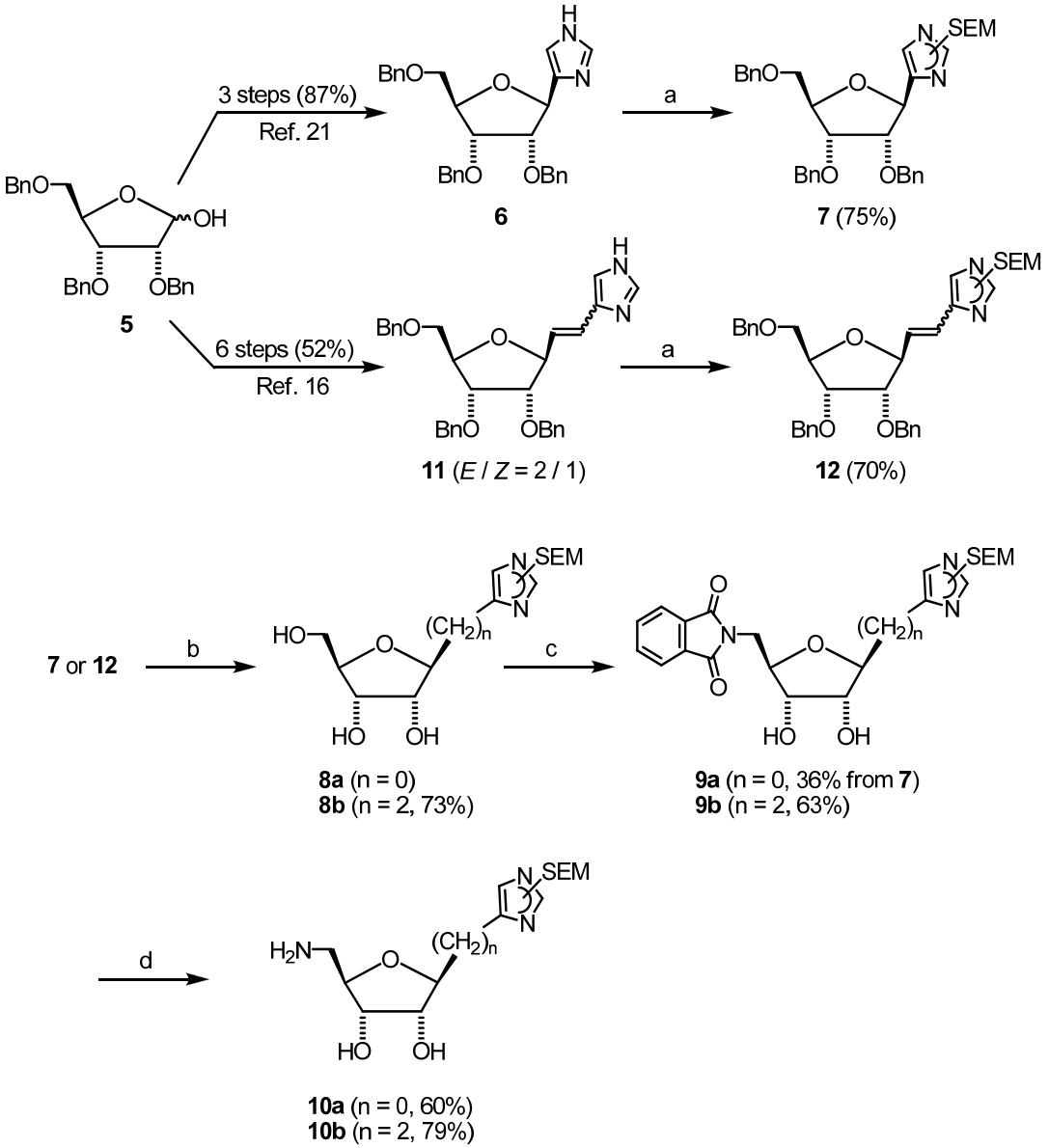


Figure 4. New E_2-imidazole *C*-nucleoside hybrid compounds

RESULTS AND DISCUSSION

The synthesis of 5'-amino imidazole C_0- and C_2-nucleosides **10a** and **10b**, each of which constitutes the right half of the target molecules **4a** and **4b**, is shown in Scheme 1. Tribenzylated β-ribofuranosyl imidazole **6** was prepared through the three steps from commercially available 2,3,5-tri-*O*-benzyl-D-ribose **5**[20] according to our previous procedures.[21] The imidazole-*N* of **6** was

protected by a [2-(trimethylsilyl)ethoxy]methyl (SEM) group to give the 3:1 isomeric mixture **7** (75%) at the endocyclic *N* functions of the imidazole. Debenzylation of **7** with $Pd(OH)_2/C$ and cyclohexene and subsequent Mitsunobu reaction [DEAD/Ph_3P/phthalimide] afforded selectively desired 5'-substituted phthalimide **9a** in 36% yield from **7**.[22] Deprotection of **9a** with hydrazine hydrate afforded 5'-amino derivative **10a** in 60% yield. By the way, we have recently reported a method to generate vinylimidazole **11** (*E*/*Z* = 2/1) from the starting material **5** by the six-step route in 52% yield.[16] Then, for the synthesis of C_2- amino homologue **10b**, vinylimidazole **11** was used as the key intermediate.[16] The introduction of a

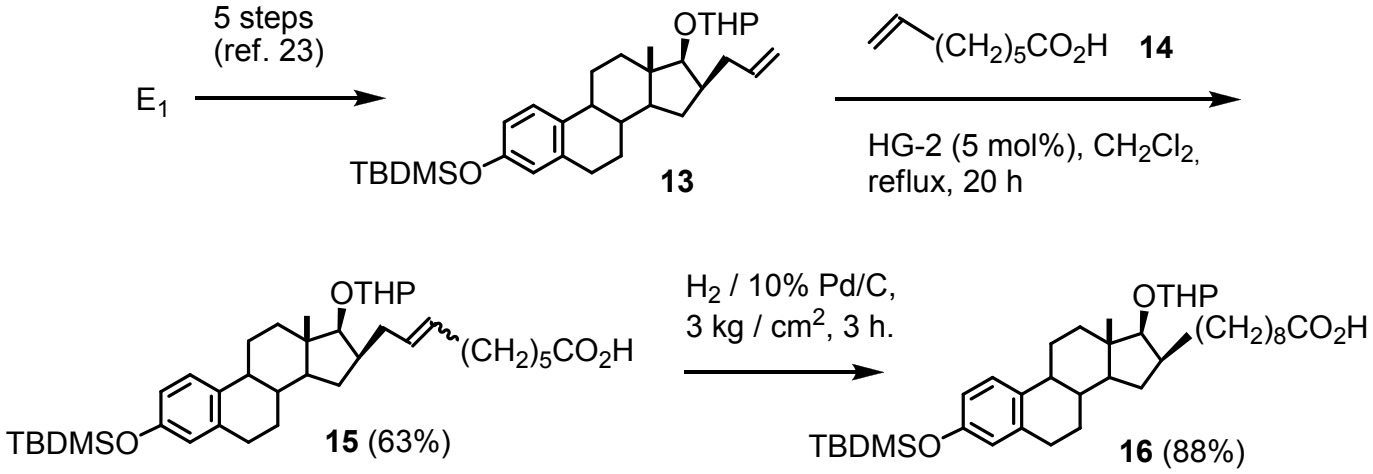


Scheme 1. Synthesis of **10a** and **10b**

Reagents and conditions: (a) NaH, SEMCl, THF, rt; (b) cyclohexene, 20% $Pd(OH)_2/C$, EtOH, reflux; (c) phthalimide, Ph_3P, DEAD, rt; d) $NH_2NH_2 \cdot H_2O$, EtOH, reflux.

SEM group at the ^{im}N position of **11** followed by debenzylation and reduction of the double bond produced *N*-SEM-imidazole C_2-ribonucleoside **8b** (73%). Conversion of **8b** into phthalimide **9b** (63%) and deprotection with hydrazine hydrate provided 5'-amino imidazole C_2-nucleoside **10b** (79%).

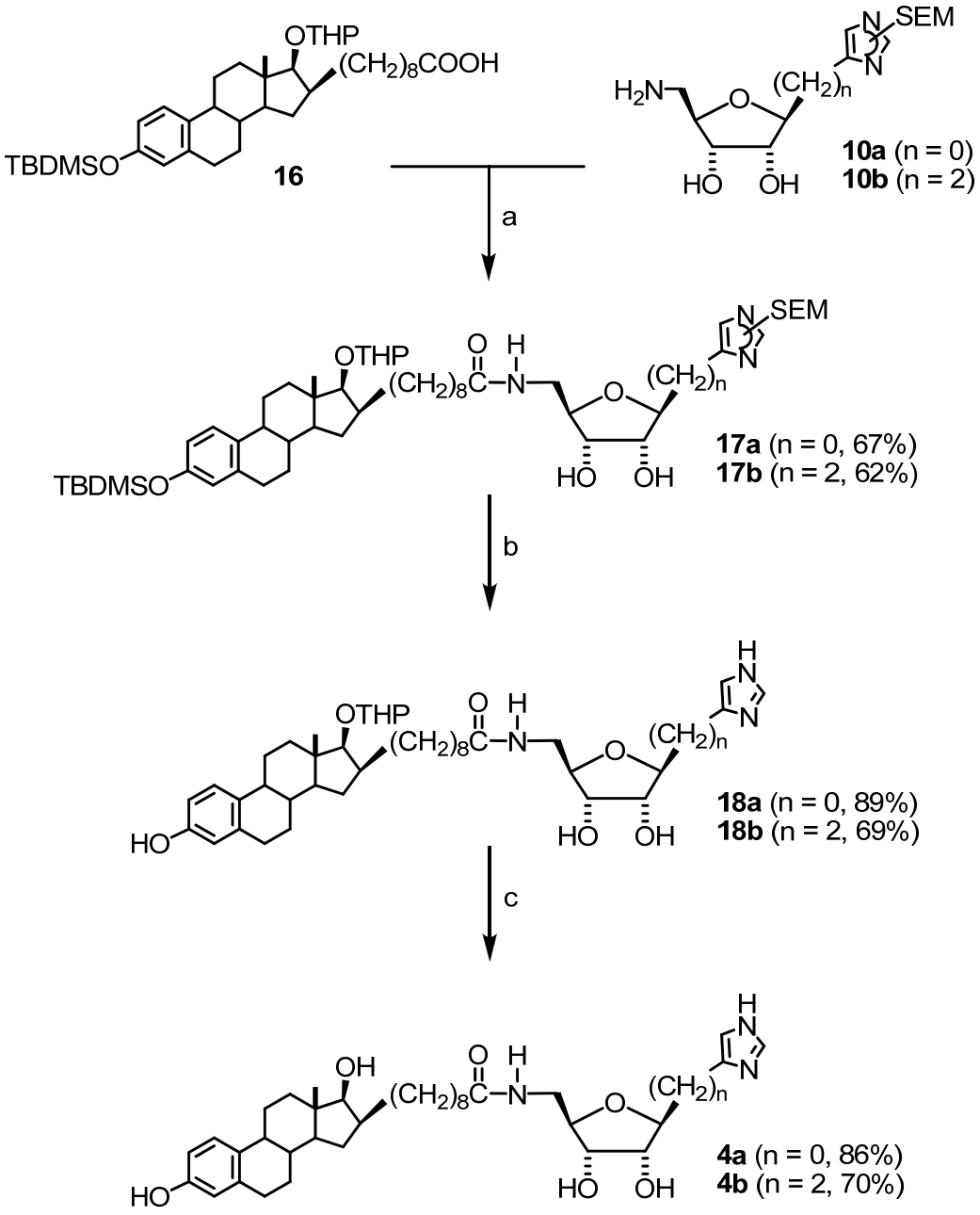
Carboxylic acid **16** as the left half was synthesized as shown in Scheme 2 and the starting protected allyl-E_2 **13** was synthesized from E_1 according to the literature.[23] Poirier *et al.* recently reported the synthesis of **16** by a cross-metathesis (CM)[13] between allyl compound **13** and 7-octenal followed by oxidation of CM product.[11b] On the other hand, as ruthenium olefin metathesis catalysts were tolerant against even the protonic functionalities like carboxylic acids and other functional groups,[24] we tried to use the commercially available 7-octenoic acid (**14**) on **13** for CM. When a mixture of allyl compound **13** and carboxylic acid **14** was refluxed in dichloromethane for 20 h in the presence of Hoveyda-Grubbs second generation catalyst (HG-2, 5 mol%), which was a powerful catalyst for CM,[25] a coupling product **15** could be obtained in 63% yield. Catalytic hydrogenation of the double bond of **15** yielded the saturated carboxylic acid **16**[11b] (88%). Direct use of carboxylic acid **14** made four steps of Poirier procedure unnecessary: three-step preparation of 7-octenal from 6-bromo-1-hexanol and oxidation of CM product.[11b]



Scheme 2. Synthesis of left half **16**

With amines **10a** and **10b** and carboxylic acid **16**, amide bond was formed in the presence of diethyl phosphorocyanidate (DEPC)[26] and triethylamine to give amide **17a** (n = 0, 67%), as shown in Scheme 3. The SEM and *tert*-butyldimethylsilyl (TBDMS) groups with tetra-*n*-butylammonium fluoride under co-existing ethylenediamine[27] were removed in refluxing THF for 2 h to give unsubstituted imidazole **18a** in 89% yield. Finally, cleavage of the tetrahydropyranyl (THP) group of **18a** with *p*-toluenesulfonic acid (*p*-TSA) successfully afforded the target C_0-compound **4a** in 86% yield. Similarly, amide compound **17b**

(n = 2), prepared from carboxylic acid **16** and C_2-amino compound **10b**, was converted into the desired C_2-hybrid compound **4b**.



Scheme 3. Synthesis of **4a** and **4b**.

Reagents and conditions: (a) DEPC, Et_3N, DMF, rt, 22 h; (b) Bu_4NF, $NH_2(CH_2)_2NH_2$, THF, reflux, 3.5 h; (c) *p*-TSA, MeOH, rt, 2 h.

Compounds **4a** and **4b** were evaluated for their ability to inhibit the *in vitro* transformation of E_1 into E_2 by a human recombinant 17β-HSD1.[28] Preliminary results were that **4b** (IC_{50}: 3.5 μM) showed more

potent 17β-HSD1 inhibitory effect than **4a** (IC_{50}: > 10 μM). In case of the ribose-$(CH_2)_n$-imidazole moiety of **4a** and **4b**, insertion of the two-methylene spacer (**4b**, n = 2) remarkably increased the inhibitory effect compared to the case of n = 0 (**4a**). Although the inhibitory potency of compound **4b** is even much lower (about 1 / 70) than EM-1745 (IC_{50}: 52 nM), such kinds of hybrid compounds as E_2-imidazole *C*-nucleoside lead to the synthesis of several analogues for a structure-activity relationship study. Further work on application of the imidazole *C*-nucleosides toward biofunctional molecules is under way and will be published in due course.

EXPERIMENTAL

Optical rotation measurements were recorded with a DIP-1000 digital polarimeter (JASCO). IR spectra were recorded on an IR-435 spectrometer (Shimadzu). ^{1}H- and ^{13}C-NMR spectra were measured with tetramethylsilane as the internal standard on Gemini-200, Mercury-300, and UNITY INOVA-500 spectrometers (Varian). Low-resolution MS and high-resolution MS were obtained on a JMS-700(2) (JEOL). Reactions with air- and moisture-sensitive compounds were carried out under the argon atmosphere. Unless otherwise noted, all extracts were dried over Na_2SO_4 and the solvent was removed in a rotary evaporator under reduced pressure. BW-127ZH and Chromatorex NH-DM 1020 [(NH-silica gel), Fuji Silysia] were used for column chromatography. Dehydrated THF was purchased (Wako). TLC was performed on the pre-coated TLC plates with $60F_{254}$ (silica gel, Merck).

4(5)-(2,3,5-Tri-*O*-benzyl-β-D-ribofuranosyl)-1-[2-(trimethylsilyl)ethoxymethyl]imidazole (7) To suspension of NaH (60%, 54 mg, 1.34 mmol) in mineral oil in THF (5 mL) was added a solution of **6**[21](420 mg, 0.89 mmol) in THF (3 ml). The mixture was stirred at room temperature (rt) for 1.5 h to stop the evolution of hydrogen. A solution of SEMCl (237 mg, 1.34 mmol) in THF (6 mL) was added to the resulting mixture. After stirring at rt for 1 h, saturated aqueous ammonium chloride was added and the whole was evaporated to give a residue, which was subsequently distributed between CH_2Cl_2 and water. After the CH_2Cl_2 layer was separated, the aqueous layer was further extracted with CH_2Cl_2. The combined CH_2Cl_2 layer was washed with water and brine, dried over anhydrous $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography on silica gel using hexane, followed by EtOAc/hexane (7/3) to give **7** (402 mg, 75%) as a pale yellow oil. The 3:1 isomeric mixture of **7** was assigned on the basis of the following ^{1}H-NMR data: ^{1}H-NMR ($CDCl_3$) δ: 0.21 (9H, s), 1.00 -1.20 (2H, m), 3.57-3.68 (2H, m), 3.68-4.00 (2H, m), 4.23-4.60 (3H, m), 4.63-4.94 (6H, m), 5.23-5.60 (3H, m), 7.18 (0.25H, s), 7.23 (0.75H, s), 7.40-7.63 (15H, m), 7.77 (1H, s). HR-MS: *m/z*: 601.3103 [Calcd for $C_{35}H_{45}N_2O_5Si$ $(M+H)^+$: 601.3095].

4(5)-(5-Deoxy-5-phthaloylamino-β-D-ribofuranosyl)-1-[2-(trimethylsilyl)ethoxymethyl]imidazole (9a) A mixture of **7** (177 mg, 0.30 mmol), 20% $Pd(OH)_2/C$ (106 mg), and cyclohexene (0.9 mL, 8.85 mmol) in EtOH (8.5 mL) was refluxed for 28 h. After filtration through Celite, the filtrate was evaporated to give 4(5)-(β-D-ribofuranosyl)-1-[2-(trimethylsilyl)ethoxymethyl]imidazole (**8a**, 96 mg). ^{1}H-NMR (CD_3OD) δ: 0.00 (9H, s), 0.91 (2H, t, *J* = 7.2 Hz), 3.50-3.82 (4H, m), 3.93-4.03 (1H, m), 4.05-4.18 (2H, m), 4.75 (1H, d, *J* = 6.0 Hz), 5.43 (1.6H, s), 5.63 (0.4 H, q, *J* = 7.0 Hz), 7.48 (1H, s), 8.35 (1H, s). HR-MS: *m/z*: 331.1685 [Calcd for $C_{14}H_{27}N_2O_5Si$ $(M+H)^+$: 331.1687]. Phthalimide (48 mg, 0.32 mmol) and Ph_3P (270 mg, 1.03 mmol) were dissolved in a solution of **8a** (96 mg) in CH_2Cl_2 (5 mL).[22,29] To this mixture, a solution of DEAD (40%, 0.47 mL, 1.03 mmol) in toluene was added slowly with stirring. After the reaction mixture was stirred for 2 h at rt, the reaction was quenched with MeOH (0.5 mL). The whole was evaporated to give a residue that was purified by column chromatography on silica gel using hexane, 50% EtOAc/hexane, EtOAc, and 10% MeOH/EtOAc to give **9a** (49 mg, 36%) as an oil. ^{1}H-NMR (CD_3OD) δ: -0.20 (9H, s), 0.78-1.00 (2H, m), 3.40-4.33 (7H, m), 4.76 (1H, d, *J* = 4.0 Hz), 5.30 (1.2H, s), 5.40 (0.8H, s), 6.90 (0.4H, s), 7.28 (0.6H, s), 7.60-7.90 (5H, m). EI-MS: *m/z*: 460 (M^++H). HR-MS: *m/z*: 460.1901 [Calcd for $C_{22}H_{30}N_3O_6Si$ $(M+H)^+$: 460.1904].

4(5)-(5-Amino-5-deoxy-β-D-ribofuranosyl)-1-[2-(trimethylsilyl)ethoxymethyl]imidazole (10a) A solution of **9a** (93 mg, 0.20 mmol) and $NH_2NH_2 \cdot H_2O$ (0.06 mL, 1.02 mmol) in EtOH (10 mL) was refluxed for 4 h and then cooled. A heaping spatula of 10% Pd/C was added to the solution and the reaction mixture was further refluxed for 20 min. After removal of the catalyst by filtration through a Celite pad, NH-silica gel (1 g) was added to the filtrate. The solvent was evaporated to give a coated silica gel, which was subsequently placed in a column (NH-silica gel, 4 g). Chromatography using 5%, 10%, and 15% MeOH in EtOAc as the eluent gave **10a** (40 mg, 60%) as an oil. ^{1}H-NMR (CD_3OD) δ: 0.00 (9H, s), 0.80-1.00 (2H, m), 2.67-3.00 (2H, m), 3.53 (2H, t, *J* = 8.0 Hz), 3.82-4.00 (2H, m), 4.15 (0.6H, t, *J* = 6.0 Hz), 4.23 (0.4H, t, *J* = 6.0 Hz), 4.72 (0.6H, d, *J* = 6.0 Hz), 4.88 (0.4H, d, *J* = 6.0 Hz), 5.33 (1.2H, s), 5.44 (0.8H, s), 7.04 (0.4H, s), 7.25 (0.6H, s), 7.80 (1H, s). ^{13}C-NMR (CD_3OD) δ: -1.4 (-1.40), -1.4 (-1.37), 18.5, 45.0, 45.1, 67.0, 67.4, 73.7, 73.8, 75.7, 76.6, 77.1, 77.2, 80.8, 85.3, 86.3, 119.1, 128.0, 132.1, 139.5, 140.9, 142.0. HR-MS: *m/z*: 330.1845 [Calcd for $C_{14}H_{28}N_3O_4Si$ $(M+H)^+$: 330.1849].

4(5)-[(*E,Z*)-2-(2,3,5-Tri-*O*-benzyl-β-D-ribofuranos-1-yl)vinyl]-1-[2-(trimethylsilyl)-ethoxymethyl]imidazole (12) Using the same procedure as that for the preparation of **7**, **11**[16] (*E*/*Z* = 2/1, 1.30 g, 2.5 mmol) was converted into the isomeric mixture **12** (1.10 g, 70%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: -0.05 (2H, s), -0.03 (3H, s), -0.01 (4H, s), 0.86 (0.4H, t, *J* = 8.1 Hz), 0.88 (0.7H, t, *J* = 8.1 Hz), 0.90

(0.9H, t, *J* = 8.1 Hz), 3.40-3.50 (2H, m), 3.52-3.65 (2H, m), 3.74 (0.2H, t, *J* = 5.3 Hz), 3.82 (0.5H, t, *J* = 5.3 Hz), 3.86 (0.3H, t, *J* = 5.3 Hz), 3.93-4.04 (1H, m), 4.22-4.30 (1H, m), 4.47-4.76 (7H, m), 5.14 (0.4H, s), 5.17 (0.6H, s), 5.20 (1H, s), 5.57 (0.3H, dd, *J* = 11.7, 8.3 Hz), 6.04 (0.2H, dd, *J* = 16.7, 6.7 Hz), 6.34 (0.5H, dd, *J* = 16.7, 6.7 Hz), 6.49 (0.3H, d, *J* = 11.7 Hz), 6.60 (0.5H, d, *J* = 16.7 Hz), 6.62 (0.2H, d, *J* = 16.7 Hz), 6.79 (0.3H, s), 7.12 (0.2H, s), 7.20-7.40 (15.5H, m), 7.55 and 7.58 (1H, 2s). HR-MS: *m/z*: 627.3252 [Calcd for $C_{37}H_{47}N_2O_5Si$ $(M+H)^+$: 627.3254].

4(5)-[2-(β-D-Ribofuranos-1-yl)ethyl]-1-[2-(trimethylsilyl)ethoxymethyl]imidazole (8b) Using the same procedure as that for the preparation of **8a**, **12** (84 mg, 0.13 mmol) was converted into **8b** (35 mg, 73%) as an oil. ^{1}H-NMR (CD_3OD) δ: -0.02 (9H, s), 0.89 and 0.90 (2H, 2t, *J* = 8.2 Hz), 1.75-2.04 (2H, m), 2.57-2.94 (2H, m), 3.50-3.61 (4H, m), 3.64-3.82 (4H, m), 5.32 (1.3H, s), 5.38 (0.7H, s), 6.90 (0.3H, s), 7.05 (0.7H, s), 7.89 (0.7H, s), 7.93(0.3H, s). HR-MS: *m/z*: 358.1919 [Calcd for $C_{16}H_{30}N_2O_5Si$ (M^+): 358.1922].

4(5)-[2-(5-Deoxy-5-phthaloylamino-β-D-ribofuranos-1-yl)ethyl]-1-[2-(trimethylsilyl)-ethoxymethyl]imidazole (9b) Phthalimide (21 mg, 0.14 mmol) and Ph_3P (89 mg, 0.34 mmol) were dissolved in a solution of **8b** (35 mg, 0.097 mmol) in THF (4 mL). To this mixture, a toluene solution of DEAD (40%, 0.15 mL, 0.34 mmol) was added slowly with stirring. The reaction mixture was stirred for 16 h at rt and then the reaction was quenched with a small amount of water. The whole mixture was evaporated to give a residue, which was subsequently purified by column chromatography on silica gel using 10% MeOH/EtOAc to give **9b** (30 mg, 63%) as a colorless oil. ^{1}H-NMR (CD_3OD) δ: -0.04 (9H, s), 0.87 (2H, t, *J* = 8.3 Hz), 1.70-1.99 (2H, m), 2.56-2.81 (2H, m), 3.42 (2H, t, *J* = 8.3 Hz), 3.72-4.13 (6H, m), 5.15 (1.3H, s), 5.17 (0.7H, s), 6.74 (0.3H, s), 6.77 (0.7H, s), 7.49 (1H, m), 7.64-7.82 (4H, m). HR-MS: *m/z*: 487.2126 [Calcd for $C_{24}H_{33}N_3O_6Si$ (M^+): 487.2137].

4(5)-[2-(5-Amino-5-deoxy-β-D-ribofuranos-1-yl)ethyl]-1-[2-(trimethylsilyl)ethoxy-methyl]imidazole (10b) Using the same procedure as that for the preparation of **10a**, **9b** (29 mg, 0.06 mmol) was converted into **10b** (17 mg, 79%) as a colorless oil. ^{1}H-NMR (CD_3OD) δ: -0.01 (9H, s), 0.84 (2H, t, *J* = 8.1 Hz), 1.72-2.06 (2H, m), 2.57-2.91 (4H, m), 3.51 and 3.53 (2H, 2t, *J* = 8.1 Hz), 3.68-3.82 (4H, m), 5.28 (0.7H, s), 5.34 (0.3H, s), 6.78 (0.3H, s), 6.96 (0.7H, s), 7.67 (1H, s). HR-MS: *m/z*: 357.2089 [Calcd for $C_{16}H_{31}N_3O_4Si$ (M^+): 357.2082].

(*E,Z*)-9-[3-(*tert*-Butyldimethylsilyloxy)-17β-(tetrahydro-2*H*-pyran-2-yl-oxy)-estra-

1,3,5(10)-trien-16*β*-yl]-7-nonenoic acid (15) A mixture of protected 16*β*-allyl E_2 **13**[23] (114 mg, 0.22 mmol), 7-octenoic acid **14** (71 mg, 0.48 mmol), and HG-2 (10 mg, 0.015 mmol) in dry CH_2Cl_2 (7 mL) was refluxed for 20 h. The solvent was evaporated to give a residue, which was subsequently chromatographed with EtOAc/hexane (10 to 30%) as eluent to give **15** (87 mg, 63%) as a foam. It was dissolved by visual bubbling in saturated aqueous sodium bicarbonate. IR (film) cm^{-1}: 1640-1810 (br, C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.19 (6H, s), 0.80 and 0.84 (3H, 2s), 0.97 (9H, s), 1.15-2.28 (30H, m), 2.35 (2H, t, *J* = 11.4 Hz), 2.72-2.86 (2H, m), 3.43-3.59 (1H, m), 3.68-3.85 (1H, m), 3.85-4.02 (1H, m), 4.61-4.79 (1H, m), 5.32-5.44 (2H, m), 6.54 (1H, d, *J* = 3.8 Hz), 6.60 (1H, dd, *J* = 12.2, 3.8 Hz), 7.10 and 7.12 (1H, 2d, *J* = 12.2 Hz). Selected ^{13}C-NMR ($CDCl_3$) δ: 117.1, 119.9, 126.1, 133.2, 137.8, 153.2, 179.5 (COOH). HR-MS: *m/z*: 624.4205 [Calcd for $C_{38}H_{60}O_5Si$ (M^+): 624.4207].

9-[3-(*tert*-Butyldimethylsilyloxy)-17*β*-(tetrahydro-2*H*-pyran-2-yl-oxy)-estra-1,3,5(10)-trien-16*β*-yl]nonanoic acid (**16**) A solution of **15** (35 mg, 0.056 mmol) in EtOH (5 mL) was hydrogenated over 10% Pd on carbon (21 mg) at 3.0 kg/cm^2 for 3 h. After filtration through Celite, a small amount of silica gel was added to the filtrate. The solvent was evaporated to give a coated silica gel, which was subsequently placed in a column. Chromatography using EtOAc/hexane (5:95) as eluent gave **16**[11b] (31 mg, 88%) as a colorless oil. It was dissolved by visual bubbling in saturated aqueous sodium bicarbonate. ^{1}H-NMR ($CDCl_3$) δ: 0.18 (6H, s), 0.79 and 0.83 (3H, 2s), 0.97 (9H, s), 1.03-2.30 (32H, m), 2.35 (2H, t, *J* = 7.0 Hz), 2.72-2.85 (2H, m), 3.44-3.56 (1H, m), 3.71 and 3.78 (1H, 2d, *J* = 9.6 Hz), 3.84-4.04 (1H, m), 4.58-4.77 (1H, m), 6.54 (1H, d, *J* = 1.9 Hz), 6.60 (1H, dd, *J* = 8.7, 1.9 Hz), 7.09 and 7.11 (1H, 2d, *J* = 8.7 Hz). HR-MS: *m/z*: 626.4359 [Calcd for $C_{38}H_{62}O_5Si$ (M^+): 626.4363].

***N*-[1-(Imidazol-4-yl)-5-deoxy-*β*-D-ribofuranos-5-yl]-9-[3-hydroxy-17*β*-(tetrahydro-2*H*-pyran-2-yloxy)-estra-1,3,5(10)-trien-16*β*-yl]nonanamide (18a)** To a solution of **16** (89 mg, 0.14 mmol) in DMF (3 mL) were added a solution of **10a** (47 mg, 0.14 mmol) in DMF (2 mL), a solution of DEPC (90%, 38 mg, 0.21 mmol) in DMF (2 mL), and Et_3N (58 μL, 0.42 mmol) in turn. The mixture was stirred at rt for 22 h and then diluted with EtOAc-hexane (3:1). The whole mixture was washed with H_2O, saturated aq. $NaHCO_3$, and brine, dried, filtered and evaporated to give a crude oil, which was purified by column chromatography (100% EtOAc to 5% MeOH in EtOAc) to give **17a** (87 mg, 67%) as an oil. ^{1}H-NMR (CD_3OD) δ: 0.00 (9H, s), 0.17 (6H, s), 0.75-0.95 (5H, m), 0.99 (9H, s), 1.03-2.33 (35H, m), 2.70-2.88 (2H, br), 3.39-4.26 (10H, m), 4.57-4.66 (0.5H, br), 4.70 (1H, d, *J* = 7.2 Hz), 4.70-4.78 (0.5H, br), 5.33 (1.3H, s), 5.45 (0.7H, s), 6.50 (1H, s), 6.57 (1H, d, *J* = 8.0 Hz), 7.03 (0.3H, s), 7.07-7.14 (1H, m), 7.26 (0.7H, s), 7.79 (0.3H, s), 7.81 (0.7H, s). HR-MS: *m/z*: 938.6116 [Calcd for $C_{52}H_{88}N_3O_8Si_2$ $(M+H)^+$:

938.6110]. Next, a 1 M solution of tetra-*n*-butylammonium fluoride (0.46 mL, 0.46 mmol) in THF and ethylenediamine (0.05 mL, 0.74 mmol) were added to a solution of **17a** (87 mg, 0.09 mmol) in THF (6 mL). The resulting mixture was refluxed for 3.5 h and then THF was evaporated to give a residue. The residue was subjected to chromatography [NH-silica gel, MeOH/$CHCl_3$ (10:90 to 20:80)] to give a pale yellow oil. As ^{1}H-NMR measurement of the oil indicated the presence of remaining tetra-*n*-butylammonium hydroxide, it was removed by filtration on C18 silica gel [MeOH/H_2O (70:30 to 100:0)] to give **18a** (57 mg, 89%) as a colorless oil. ^{1}H-NMR (CD_3OD) δ: 0.77 (1.2H, s), 0.82 (1.8H, s), 0.86-2.30 (35H, m), 2.72-2.80 (2H, br), 3.18-4.12 (8H, m), 4.57-4.62 (0.5H, br), 4.70-4.75 (0.5H, br), 4.75 (1H, d, *J* = 7.2 Hz), 6.46 (1H, s), 6.52 (1H, d, *J* = 8.0 Hz), 7.02-7.08 (1H, m), 7.10 (1H, s), 7.71 (1H, s). HR-MS: *m/z*: 694.4428 [Calcd for $C_{40}H_{60}N_3O_7$ $(M+H)^+$: 694.4431].

***N*-[1-(Imidazol-4-yl)-5-deoxy-β-D-ribofuranos-5-yl]-9-[3,17β-dihydroxy-estra-1,3,5(10)-trien-16β-yl]nonanamide (4a)** *p*-TsOH (15 mg, 0.09 mmol) was added to a solution of **18a** (56 mg, 0.08 mmol) in MeOH (3.5 mL). After stirring at rt for 2 h, the reaction mixture was neutralized with saturated aq. $NaHCO_3$ and evaporated. The residue was dissolved in EtOAc and the solution was washed with saturated aq.$NaHCO_3$ and brine, dried, filtered, and evaporated. The residual oil was purified on N-H silica gel using MeOH-$CHCl_3$ (5:95 to 15:85, v/v) to give **4a** (42 mg, 86%) as a colorless oil. Rf = 0.33 (30% MeOH in $CHCl_3$). $[\alpha]_D$ = + 26.2^o (*c* = 1.85, MeOH). ^{1}H-NMR (CD_3OD) δ: 0.76 (3H, s), 0.93-2.30 (29H, m), 2.71-2.84 (2H, m), 3.20-3.70 (3H, m), 3.93-3.99 (2H, m), 4.06-4.11 (1H, m), 4.73 (1H, d, *J* = 6.1 Hz), 6.47 (1H, d, *J* = 2.7 Hz), 6.53 (1H, dd, *J* = 8.5, 2.7 Hz), 7.06 (1H, d, *J* = 8.5 Hz), 7.09 (1H, s), 7.68 (1H, s). ^{13}C-NMR (CD_3OD) δ: 13.3, 27.1, 27.6, 28.8, 29.9, 30.4, 30.5, 30.7, 30.8, 31.0, 33.0, 33.7, 37.1, 39.1, 40.0, 41.7, 42.5, 45.2, 45.5, 49.9, 50.0, 62.3, 73.5, 76.8, 80.2, 83.4, 83.8, 113.7, 116.1, 127.2, 132.7, 137.0, 138.8, 155.9, 176.7. HR-MS: *m/z*: 610.3860 [Calcd for $C_{35}H_{52}N_3O_6$ $(M+H)^+$: 610.3856].

***N*-{1-[2-(Imidazol-4-yl)ethyl]-5-deoxy-β-D-ribofuranos-5-yl}-9-[3-hydroxy-17β-(tetrahydro-2*H*-pyran-2-yloxy)-estra-1,3,5(10)-trien-16β-yl]nonanamide (18b)** A mixture of **16** (67 mg, 0.11 mmol), **10b** (41 mg, 0.12 mmol), DEPC (29 mg, 0.16 mmol), and Et_3N (0.05 mL, 0.32 mmol) in DMF (10 mL) was stirred at rt for 13 h to give **17b** (64 mg, 62%) as a colorless oil using the same procedure as that for the preparation of **17a**. ^{1}H-NMR (CD_3OD) δ: -0.08 (9H, s), 0.17 (6H, s), 0.78 and 0.82 (3H, 2s), 0.84-0.93 (2H, m), 0.96 (9H, s), 1.02-2.06 (33H, m), 2.08-2.30 (2H, m), 2.18 (2H, t, *J* = 6.9 Hz), 2.62-2.88 (4H, m), 3.36-3.58 (5H, m), 3.66-3.88 (5H, m), 3.89-4.15 (1H, m), 4.57-4.75 (1H, m), 5.18 (0.7H, s), 5.21 (0.3H, s), 6.50 (1H, d, *J* = 2.3 Hz), 6.55 (1H, dd, *J* = 9.2, 2.3 Hz), 6.78 (0.3H, s), 6.94

(0.7H, s), 7.08 and 7.10 (1H, 2d, *J* = 9.2 Hz), 7.52 (1H, s). HR-MS: *m/z*: 966.6422 [Calcd for $C_{54}H_{92}N_3O_8Si_2$ $(M+H)^+$: 966.6418]. A mixture of **17b** (18 mg, 0.02 mmol), 1 M THF solution of tetra-*n*-butylammonium fluoride (0.09 mL, 0.09 mmol) and ethylenediamine (0.01 mL, 0.15 mmol) in THF (1.5 mL) was refluxed for 2 h to give **18b** (9 mg, 69%) as a colorless oil using the same procedure as that for the preparation of **18a**. ^{1}H-NMR (CD_3OD) δ: 0.78 and 0.82 (3H, 2s), 1.08-2.06 (37H, m), 2.06-2.83 (2H, m), 2.20 (2H, t, *J* = 8.2 Hz), 2.60-2.83 (4H, m), 3.26-3.56 (2H, m), 3.64-3.82 (5H, m), 3.84-4.03 (1H, m), 4.56-4.73 (1H, m), 5.32-5.43 (2H, m), 6.46 (1H, d, *J* = 3.0 Hz), 6.52 (1H, dd, *J* = 9.0, 3.0 Hz), 6.77 (1H, s), 7.04 and 7.05 (1H, 2d, *J* = 9.0 Hz), 7.53 (1H, s). HR-MS: *m/z*: 722.4739 [Calcd for $C_{42}H_{64}N_3O_7$ $(M+H)^+$: 722.4740].

***N*-{1-[2-(Imidazol-4-yl)ethyl]-5-deoxy-β-D-ribofuranos-5-yl}-9-[3,17β-dihydroxyestra-1,3,5(10)-trien-16β-yl]nonanamide (4b)** A mixture of **18b** (20 mg, 0.03 mmol) and *p*-TsOH (5 mg, 0.03 mmol) in MeOH (1 mL) was stirred at rt for 4 h to give **4b** (12 mg, 70%) as a colorless oil using the same procedure as that for the preparation of **4a**. Rf = 0.32 (20% MeOH in $CHCl_3$). $[\alpha]_D$ = + 25.5° (*c* = 1.65, MeOH). ^{1}H-NMR (CD_3OD) δ: 0.76 (3H, s), 1.22-2.34 (32H, m), 2.20 (2H, t, *J* = 7.5 Hz), 2.60-2.87 (4H, m), 3.64-3.83 (5H, m), 6.46 (1H, d, *J* = 3.2 Hz), 6.52 (1H, dd, *J* = 7.9, 3.2 Hz), 6.78 (1H, s), 7.06 (1H, d, *J* = 7.9 Hz), 7.56 (1H, s). ^{13}C-NMR (CD_3OD) δ: 13.5, 24.1, 27.2, 27.7, 28.9, 30.0, 30.5, 30.6, 30.8, 30.9, 31.2, 33.1, 33.7, 34.9, 37.1, 39.1, 40.1, 41.7, 42.8, 45.2, 45.5, 50.0, 73.8, 76.0, 83.1, 83.3, 113.2, 115.5, 126.6, 132.1, 135.1, 138.1, 155.1, 175.6. HR-MS: *m/z*: 638.4165 [Calcd for $C_{37}H_{56}N_3O_6$ $(M+H)^+$: 638.4166].

Biological Assay

Human 17β-HSD1 was overexpressed in the BL21-AI strain of *Escherichia coli* containing the pET-41 Ek/LIC-17β-HSD1 vector, basically according to the method of Chang *et al*.[28] The human recombinant 17β-HSD1 was purified by using a Glutathione Sepharose 4B column (GE Healthcare) and a Mono Q 5/50 GL column (GE Healthcare). The purified enzyme was incubated with substrate E_1 and cofactor NADH in the presence or absence of inhibitors at pH 7.4. The residual E_1 and newly produced E_2 in the incubation mixture were extracted with hexane. The organic phase was evaporated, the residue containing E_1 and E_2 was dissolved in acetonitrile solution and then measured by reverse-phase high-performance liquid chromatography with an amperometric detector (HTEC-500, EICOM).

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